



UNIVERSITI PUTRA MALAYSIA

**EFFECT OF *CHANNA STRIATUS* AND *CURCUMA LONGA* IN
EXPERIMENTALLY-INDUCED OSTEOARTHRITIS IN RABBITS**

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By

MICHELLE NG YEEN TAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Master of Science**

July 2003



Specially dedicated to my parents, Weng Onn, my two elder brothers and sisters-in-law and not forgetting my adorable nephews and nieces.

I love you all very much!
Thanks for your love, encouragement and support!

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirements for the degree of Master of Science

**EFFECT OF *CHANNA STRIATUS* AND *CURCUMA LONGA* EXTRACTS IN
EXPERIMENTALLY-INDUCED OSTEOARTHRITIS IN RABBITS**

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July 2003

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Channa striatus and *Curcuma longa* are two well known natural products that have long been used in treating various kinds of ailments. *Channa striatus* is high in essential amino acids and fatty acids that played an important role in wound healing as well as in anti-nociceptive activities. *Curcuma longa* on the other hand, contains an active compound called curcuminoids that are responsible for its anti-inflammatory, anti-oxidant and anti-cancer properties.

Therefore, in this study, *Channa striatus* and *Curcuma longa* extracts were used in the treatment of experimentally induced Osteoarthritis (OA) in rabbits. OA was induced on the right stifle joint of the rabbits in the treatment and the negative control groups by transecting the anterior cruciate ligaments.

These animals were left for 8 weeks to develop OA. Radiography and ultrasonography were performed on the induced joints to determine the development of OA prior to *Channa striatus* and *Curcuma longa* treatments.

During the progression of OA, the induced joints began to show sign of OA development as early as the 2nd week after induction of OA as observed in ultrasonograph. Slight joint space narrowing, which reflect the deteriorating articular cartilage was detected by the ultrasonography as early as the 2nd week post induction. On the 3rd week after the induction of OA, ultrasonography was able to detect significant joint space narrowing and total diminution of joint space on the 4th week post induction. In addition to that, irregular joint surface has developed in the induced joints as seen on the ultrasonograph taken on the 5th and the 6th week post induction. Apart from these, other structures such as the infrapatellar fat, the patellar ligament and the synovial membrane in the induced joints also underwent osteoarthritic changes as seen in ultrasonographs.

On the 8th week post induction there was a significant periarticular soft tissue swelling detected by radiography and ultrasonography. Soft tissue swelling detected on the radiographs was seen as an increased radiopacity area around the joint. In ultrasonographs, the swelling of the joint could be observed as an increased distance between the surface of joint and the skin compared to the normal uninduced joints. On the 9th week of treatment, a significant reduction of soft tissue swelling was observed on *Channa striatus*- and *Curcuma longa*- treated joints compared to the untreated joints.

Although the treatments were effective in reducing inflammations and swelling, these extracts did not exhibit any improvement on other structures of the joints. Extra bone formation and diminution of the joint space were observed on both radiographs and ultrasonographs on the 9th week of treatment. These similar changes were further confirmed with the gross findings on the opened joints upon euthanasia.

In the immunohistochemistry study, synovial membrane biopsies from the normal, treated and negative control joints were obtained to study the general innervation of the synovial membrane. The immunoreactive fibres stained against PGP 9.5, CGRP and NPY antisera were not detected in the control joints compared to the normal synovial membrane. The synovial membrane from the untreated joints was heavily infiltrated with inflammatory cells, which may account for the diminished immunoreactive nerve fibres from the synovial membrane.

However, the number of immunoreactive nerve fibres detected in the synovial membranes from *Channa striatus* and *Curcuma longa* treated joints was higher than in the synovial membrane from the control untreated joint. They exhibited a similar distribution to the nerve fibres found in normal synovial membrane but less numerous.

Therefore, the present study showed that both *Channa striatus* and *Curcuma longa* extracts showed good signs of healing in OA and these extracts can be used as a good alternative treatment in OA.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN EKSTRAK *CHANNA STRIATUS* DAN *CURCUMA LONGA* DI
DALAM UJIKAJI OSTEOARTRITIS TERARUH PADA LUTUT ARNAB**

Oleh

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Channa striatus dan *Curcuma longa* merupakan dua produk semulajadi yang telah lama digunakan dalam mengubati pelbagai jenis penyakit. *Channa striatus* mengandungi asid amino perlu dan asid lemak yang tinggi, yang memainkan peranan penting dalam menyembuhkan luka dan mengurangkan kesakitan. *Curcuma longa* pula terdiri daripada curcuminoids yang bertanggungjawab dalam kesan anti-inflammatori, anti oksidan dan anti- kansernya.

Oleh itu, ekstrak *Channa striatus* dan *Curcuma longa* telah digunakan dalam ujikaji ini untuk merawat OA teraruh dalam lutut arnab. Di dalam ujikaji ini, osteoarthritis telah diaruhkan pada lutut kanan arnab daripada kumpulan rawatan dan kawalan negatif, dengan memotong ligamen krusiat anterior. Arnab-arnab ini kemudiannya dibiarkan selama 8 minggu supaya OA dapat

berkembang. Sementara itu, pemeriksaan radiograf dan ultrasonograf telah dijalankan pada lutut yang diaruh untuk memastikan penyakit ini telah berkembang pada lutut-lutut ini sebelum rawatan *Channa striatus* dan *Curcuma longa* dimulakan.

Semasa perkembangan OA, lutut-lutut teraruh ini mula menunjukkan perubahan-perubahan osteoartritik seawal-awal 2 minggu selepas aruhan OA. Penyusutan kecil ruang di antara sendi yang menandakan kerosakan pada sendi tulang rawan telah dapat dikesan oleh ultrasonografi seawal-awal 2 minggu selepas aruhan. Penyusutan ruang antara sendi yang ketara telah dikesan pada minggu yang ke-3 dan pada minggu ke-4 selepas aruhan, tiada lagi ruang di antara sendi telah dikesan. Tambahan pula, ketidakrataan permukaan pada sendi telah dilihat pada ultrasonograf pada minggu yang ke-5 dan ke-6 selepas aruhan. Di samping itu, struktur-struktur lain dalam sendi lutut teraruh seperti lemak infrapatela, ligamen patela dan membran sinovial juga telah menunjukkan perubahan-perubahan osteoartritik seperti yang dapat dilihat pada ultrasonograf.

Pada minggu ke-8 selepas aruhan OA, pembengkakan tisu lembut di sekitar sendi telah dapat dikesan oleh radiografi dan ultrabunyi. Pembengkakan tisu lembut dapat dilihat pada radiograf sebagai peningkatan 'radiopacity' di sekitar sendi manakala pada ultrasonograf, bengkak tisu lembut pada sendi dapat dilihat sebagai peningkatan jarak di antara permukaan sendi dan kulit. Walaubagaimanapun, bengkak pada tisu lembut

dalam rawatan ekstrak *Channa striatus* dan *Curcuma longa* telah susut pada minggu ke-9 rawatan berbanding dengan lutut-lutut yang tidak dirawat.

Walaupun rawatan yang diberikan berkesan dalam mengurangkan bengkak pada tisu lembut, ia adalah kurang berkesan dalam menghindari perubahan struktur lain dalam sendi. Pertumbuhan tulang tambahan dan penyusutan sepenuh ruang di antara sendi dalam lutut-lutut daripada kumpulan rawatan dapat dilihat pada radiograf dan ultrasonograf pada minggu ke-9 rawatan. Perubahan-perubahan sedemikian juga dapat dilihat dengan mata kasar melalui bedah siasat lutut-lutut ini selepas eutanasia.

Di dalam ujikaji immunohistokimia pula, membran sinovial daripada kumpulan normal, rawatan dan kawalan telah diperoleh untuk mengkaji keseluruhan rangkaian gentian saraf. Secara keseluruhannya, gentian immunoreaktif yang dilabel oleh antisera PGP 9.5, CGRP dan NPY tidak dapat dikesan pada membran sinovial daripada kumpulan kawalan negatif berbanding kumpulan normal. Didapati juga, membran sinovial daripada kumpulan kawalan dipenuhi dengan sel-sel inflamatori yang mungkin menyebabkan kehilangan gentian-gentian immunoreaktif daripada membran sinovial .

Walaubagaimanapun, bilangan gentian saraf di dalam membran sinovial daripada kumpulan rawatan *Channa striatus* dan *Curcuma longa* adalah lebih tinggi berbanding dengan membran sinovial daripada kumpulan kawalan negatif. Didapati gentian-gentian saraf ini menyerupai distribusi

gentian saraf dalam membran sinovial daripada kumpulan normal, tetapi bilangannya adalah kurang.

Oleh itu, ekstrak *Channa striatus* and *Curcuma longa* ini telah menunjukkan kesan-kesan positif dalam rawatan OA dan dengan demikian ekstrak-ekstrak ini boleh digunakan sebagai rawatan alternatif yang baik dalam rawatan OA.

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I certify that an Examination Committee met on 10th July 2003 to conduct the final examination of Michelle Ng Yeen Tan on her Master of Science thesis entitled "Effect of *Channa striatus* and *Curcuma longa* in Experimentally-Induced Osteoarthritis in Rabbits" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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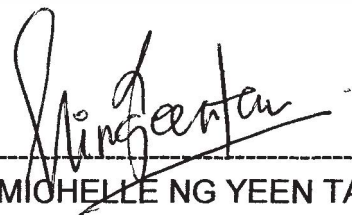
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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



(MICHELLE NG YEEN TAN)

Date: 15/8/03

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LIST OF ABBREVIATIONS

ABPC	Avidin biotinylated peroxidase complex
ACL	Anterior cruciate ligament
CGRP	Calcitonin gene-related peptide
DAB	Diaminobenzidine
H&E	Haematoxylin-eosin
HA	Hyaluronic acid
IgG	Immunoglobulin G
mg/kg	milligram/kilogram
MHz	MegaHertz
MRI	Magnetic resonance imaging
NGF	Nerve growth factor
NPY	Neuropeptide Y
NSAIDs	Non-steroidal anti-inflammatory drugs
OA	Osteoarthritis
PBS	Phosphate buffer saline
PGP 9.5	Protein gene product 9.5
RA	Rheumatoid arthritis
ROS	Reactive oxygen species
SP	Substance P
w:v	Weight : volume
μ M	Micrometer